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Serologic Survey for Leptospirae in European Brown Bears (*Ursus arctos*) in Croatia

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ABSTRACT: From 1981 to 1991, sera of 42 European brown bears (*Ursus arctos*) from three areas in Croatia were tested for antibodies against 12 *Leptospira interrogans* serovars: *grippotyphosa, sejroe, australis, pomona, canicola, icterohaemorrhagiae, tarassovi, saxkoebing, ballum, bataviae, poi,* and *hardjo*. Diagnostic levels of antibody were found in 17 (40%) of 42 sera. Evidence of exposure to at least one of the serovars was found in seven of 14 free-ranging bears from the Lika region, four of 12 free-ranging bears from the Gorski Kotar region, zero of six orphaned cubs from the Gorski Kotar region, and six of 10 captive bears from the Zagreb Zoo. Based on the antibody titers, we implicated the following serovars: *australis* in five bears, *sejroe* in two bears, *canicola* in one bear, and *icterohaemorrhagiae* in one bear. There was a strong correlation between serovars implicated by this survey and serovars previously isolated from small mammals in Croatia.

Key words: European brown bear, *Ursus arctos*, leptospirosis, *Leptospira interrogans*, serovars, serology, survey, Croatia.

There are few reports of antibodies against leptospirae in bears. Isolation of leptospirae or the evidence of the disease in bears have not been reported. Kleinschmidt (cited in Kathe and Mochmann, 1967) found antibodies against *Leptospira interrogans* serovar *icterohaemorrhagiae* in a captive circus bear in Germany. Antibodies against serovar *pomona* (1:400) and serovar *autumnalis* (1:200) were found in a 2-yr-old female American black bear (*Ursus americanus*) and against serovar *pomona* (1:1,600) and serovar *icterohaemorrhagiae* (1:400) in a female cub from Pennsylvania (USA) (Matula et al., 1980). Two of 196 American black bears from Idaho (USA) had low antibody titers to serovar *grippotyphosa* (Binninger et al., 1980). In Gorski Kotar in Croatia, Karlović et al. (1985) found antibodies in three of ten European brown bears (*U. arctos*). One each was positive to serovar *grippotyphosa, saxkoebing* or *sejroe*. Karlović et al. (1990) reported on 73 brown bear sera from Croatia; nine of 44 from Gorski Kotar, two of eight from Lika, and one of seven from Hrvatsko primorje were positive.

Approximately 400 brown bears are believed to inhabit Croatia (Huber and Morić, 1989). They are part of the Dinara Mountains population which is the largest in south-western Europe and is the source for reintroductions to other countries. Thus the knowledge about the health status of Croatian bears is an important management factor. Our objectives were to determine the prevalence of antibodies against leptospirae among bears in Croatia, the serovars with which the bears come in contact, possible sources of infection for bears, and any evidence of disease in bears.

Blood samples were taken from 22 male and 20 female European brown bears. Twenty-six (18 males and eight females) were sampled during capture for radiotagging in Croatia. Fourteen were captured in Lika (44°55′N, 15°39′E) and 12 in Gorski Kotar (45°27′N, 14°38′E). Age was estimated by counting cementum layers around the root of the extracted rudimentary first premolar tooth (Stonenberg and Jonkel, 1966). The mean age of the free-living bears was 5.0 years (SD = 2.9, range 1 to 12 years). An additional six bears (one male and five females) were 1 to 3-mo-old orphaned cubs, originating from Gorski Kotar. The remaining seven bears (three males and four females) were from the Zagreb Zoo, with a mean age of 7.2 yr (SD = 4.3, range 0.4–14 years). Blood samples were taken from the femoral vein or artery during chemical immobilization with ketamine 11mg/kg hydrochloride (Ketalar,

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TABLE 1. Reciprocal titers of *Leptospira interrogans* antibody in sera of European brown bears from Croatia and the frequency of highest titers. Only the 17 positive bears are listed.

Bear identification[a]	Sex	Age (yr)	Mass (kg)	Date of sampling	*Leptospirae* serovar[b]											
					gri.	*sej.*	*aus.*	*pom.*	*can.*	*ict.*	*tar.*	*sax.*	*bal.*	*bat.*	*poi.*	*har.*
L1	f[c]	1	39	Nov 1981	–	100	–	–	–	–	–	–	–	–	–	–
L5	m	7	222	Sep 1984	–	–	100	–	–	–	–	–	–	–	100	–
L8	m	1	20	Apr 1985	–	500	–	–	–	–	–	100	–	–	–	–
L9	m	5	119	May 1986	–	–	100	–	–	–	–	–	–	–	–	–
L11	m	5	86	May 1986	–	–	100	–	–	–	–	–	–	–	–	–
L13	m	5	111	Apr 1988	–	100	100	–	–	–	–	100	–	–	100	100
L14	m	3	59	Apr 1988	–	–	100	–	–	–	–	–	–	–	–	–
G4	m	5	127	May 1989	–	100	100	–	–	–	–	–	–	–	–	–
G8	m	4	120	Oct 1989	–	100	2,000	–	–	–	–	500	–	–	–	1,000
G9	f	13	153	Oct 1989	–	–	100	–	–	–	–	–	–	–	–	–
G11	f	2	43	Jun 1990	–	100	–	–	–	–	–	100	–	–	–	–
Z2	f	3	47	Nov 1986	–	–	100	–	100	1,000	–	–	–	–	100	–
Z5	f	4	120	Apr 1990	–	–	–	–	100	–	–	–	–	–	–	–
Z10	f	8	103	Mar 1991	–	–	100	–	100	100	–	–	–	–	–	–
Z11	f	14	138	May 1992	–	–	–	–	–	100	–	–	–	–	100	–
Z13	m	8	100	May 1991	–	–	–	–	100	100	–	–	–	–	100	–
Z14	f	7	97	Jun 1991	100	–	100	–	–	500	–	–	500	–	–	–
Frequency of having the highest titer						2	5		1	1						

[a] L = Lika; G = Gorski Kotar; Z = Zagreb Zoo.

[b] *gri.* = *grippotyphosa*, *sej.* = *sejroe*, *aus.* = *australis*, *pom.* = *pomona*, *can.* = *canicola*, *ict.* = *icterohaemorrhagiae*, *tar.* = *tarassovi*, *sax.* = *saxkoebing*, *bal.* = *ballum*, *bat.* = *batavie*, *poi.* = *poi*, *har.* = *hardjo*.

[c] f = female; m = male.

Parke-Davis, Berlin, Germany) and 6 mg/kg xylazine hydrochloride (Rompun, Bayer, Leverkusen, Germany). After separation, serum was stored at −18 C.

The microscopic agglutination test (Gochenour et al., 1958) was performed for 12 *L. interrogans* serovars: *grippotyphosa*, *sejroe*, *australis*, *pomona*, *canicola*, *icterohaemorrhagiae*, *tarassovi*, *saxkoebing*, *ballum*, *bataviae*, *poi*, and *hardjo*. The initial serum dilution was 1:100; positive sera were further diluted to the end-point of 50% agglutination. Microagglutination titers of ≥1:100 were considered as indicative of previous exposure. If sera were positive to two or more leptospirae serovars, the serovar with the highest antibody titer was interpreted to be the serovar that caused infection. However, occasionally cross-reactivity among serovars may result in a titer that may be the same or even higher (Modrić et al., 1985). For eight sera that had equal titers to more than one serovar, we did not ascribe one serovar as the source of infection. Chi-squared tests (Burington and May, 1958) were used for statistical comparisons; $P < 0.05$ was considered as a statistically significant difference.

We found antibodies against leptospirae in 17 (40%) of 42 European brown bear sera, with reciprocal titers ranging from 100 to 2,000 (Table 1). Antibodies against leptospirae were found in seven of 14 samples from Lika, in four of 12 from Gorski Kotar, and in six of 10 from the Zagreb Zoo. All six bear cubs found in Gorski Kotar were negative. Highest antibody titers were to serovar *australis* in five bears, *sejroe* in two, and *canicola* and *icterohaemorrhagiae* each in one serum. In eight sera we found the same antibody titer for two to five serovars (Table 1). No bears showed evidence of disease at the time of sampling.

The 40% antibody prevalence against leptospirae we found in Croatia is the

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highest published for bears. Earlier Karlović et al. (1985, 1990) reported 15 (18%) of 83 bears positive. Their lower prevalence may be explained by their use of the paper strips with dried blood which is a less sensitive method. Comparison of our results with the earlier studies (Karlović et al., 1985, 1990) is also limited because their method did not permit ranking the frequency of titers.

The prevalence of antibodies in captive bears (six positive of 10) was higher than in free-living and free-born animals (11 positive of 32), but the difference was not statistically significant. There were no significant differences in antibody prevalence between the bears from Lika and Gorski Kotar, or between age and sex classes.

Antibody titers against serovar *australis* were highest in five bears (Table 1). In Croatia, serovar *australis* is a cause of leptospirosis in humans (Zaharija, 1955) and has been isolated from the kidney of a clinically healthy cat (Modrić, 1978). Borčić et al. (1982) isolated serovar *australis* from four species of small mammals (*Apodemus agrarius, A. sylvaticus, A. flavicollis* and *Clethrionomys glareolus*) in Croatia. Four other leptospirae serovars (*grippotyphosa, pomona, sejroe*, and *bataviae*) also were isolated from 15 small mammal species. Thus, small mammals may be potential sources of infection with serovar *australis* for other animals, including European brown bears.

Antibodies against serovar *sejroe* were highest in two bear sera. Borčić et al. (1982) also found evidence for infection of bears by small mammals.

Antibodies against serovar *icterohaemorrhagiae* in the Zagreb Zoo bear may have been due to infection from rats (*Rattus norvegicus*), the main reservoir of this leptospirae serovar in Croatia (Zaharija and Perić, 1969). Antibodies against serovar *canicola* may reflect contact with dogs, the only documented reservoir of serovar *canicola* in Croatia (Zaharija, 1954). Free-living rats and mice and stray dogs are practically the only mammals which can get in contact with caged bears in the Zagreb Zoo.

Bears are opportunistic feeders that forage on and below ground. Mammalian protein comprises only a small proportion of the European brown bear's diet (<1% diet volume and 4% frequency of occurrence in scats), but the share of small mammals in protein is 75% (Cicnjak et al., 1987). We speculate that the leptospirae infections in bears depend on distribution of this agent among small mammals. The high prevalence of antibodies against leptospirae in bears in Croatia are evidence for the circulation of certain leptospire serovars in their population. The absence of clinical cases in our sample does not exclude the possibility of subclinical or past infections.

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